

**REMARKS**

Reconsideration of this application is respectfully requested. The specification has been amended to include the priority information for this application, and to italicize the term *Mycobacterium w*.

Claims 37, 48-50, 66, and 69 have been amended to more specifically recite the components of the pharmaceutical composition. Support for this amendment is found in the specification at, e.g., pages 5-7 and 10 of the specification. Claim 37 has been further amended to remove the term “managing.” Claims 38-39, 42, 58, and 68 have been amended for clarification. Support for these amendments is found in the specification at, e.g., page 12, line 8 to page 13, line 9, and page 13, line 20 to page 14, line 19. Claims 57, 61-63, and 65 have been amended to correct clerical errors. Claim 69 has been further amended to recite specific symptoms to be ameliorated. No new matter has been added by way of this amendment. Claims 37-69 are pending and at issue.

**Objections to the Specification**

The specification has been objected to for inconsistent reference to the term “*Mycobacterium w*.” The specification has been amended as suggested by the Examiner so the term “*Mycobacterium w*” is italicized throughout.

The specification has also been objected to for containing various typographical errors. For clarification, these errors have been corrected as indicated in the presently submitted substitute specification.

### **Indefiniteness Rejection**

Claims 37-69 have been rejected as indefinite because the term “Mycobacterium w” is not properly capitalized. Claim 1 has been amended to replace each reference to “mycobacterium w” with “*Mycobacterium w*”.

Claim 37 has been rejected as indefinite for reciting “managing cancer”. According to the Examiner, it is unclear how the present composition would “manage” the disease. While applicants respectfully disagree, in order to advance prosecution, this term has been removed from claim 37.

Claims 37 and 48-51 have been rejected as indefinite for reciting “constituents of mycobacterium w.” According to the Examiner, it is unclear what is encompassed by the term “constituents”. While applicants respectfully disagree, in order to advance prosecution, this phrase has been deleted from the claims.

Claim 38 has been rejected as indefinite because the term “decreasing the burden of cancer tissue” is allegedly ambiguous. The claim has been amended for clarification to recite that the method is for “reducing progression of cancer tissue”.

Claims 39 and 40 have been rejected for reciting methods of managing the effects of cancer therapy. While applicants respectfully submit that these claims are definite, in order to advance

prosecution, claim 39 has been amended to recite that the method is for “increasing the efficacy of radiotherapy or chemotherapy in the treatment of cancer.”

Claim 42 has been rejected for reciting a method to “avoid postponement of chemotherapy” because it depends from a claim in which chemotherapy is performed. For clarification, the claim has been amended to recite that the side-effects are reduced to “avoid interruption of chemotherapy”.

Claim 57 has been rejected for containing typographical errors. Claim 57 has been amended as suggested by the Examiner to correct the inaccurate spelling of “hydrolyze”.

Claim 58 has been rejected as indefinite for reciting “other modes of therapy”. While applicants respectfully submit that these claims are definite, in order to advance prosecution, claim 58 has been amended to recite that the other modes of therapy are “selected from radiotherapy and chemotherapy

Claim 65 has been rejected because the term “metastatic” is enclosed in parentheses. According to the Examiner, it is unclear whether this term is intended to be part of the claim. For clarification, claim 65 has been amended to remove the parentheses.

Claim 66 has been rejected as indefinite for containing a typographical error. Claim 66 has been amended as suggested by the Examiner to replace the term “quality of like” with “quality of life”.

Claim 68 has been rejected because according to the Examiner it is unclear what is encompassed by the phrase “with the addition of other modes of treatment”. While applicants respectfully disagree, in order to advance prosecution, claim 68 has been amended to recite that the other modes of therapy are “selected from radiotherapy and chemotherapy”.

Claim 69 has been rejected by the Examiner because it is unclear what is encompassed by the term “symptoms associated with cancer”. While applicants respectfully disagree, in order to advance prosecution, claim 69 has been amended to recite that the symptoms associated with cancer are “selected from pain, abnormal hemoglobin, cough, breathlessness, dysphagia, neutropenia, and irregular sleep”.

For the reasons above, the pending claims are definite and applicants respectfully request withdrawal of this rejection.

#### **Enablement Rejection**

Claims 37-69 have been rejected for lacking enablement. The Examiner contends that the specification fails to sufficiently disclose certain descriptive features of the *Mycobacterium w* preparations. Specifically, the Examiner contends that the specific dosage administered, the route of administration, and frequency of administration are not provided, and that it is unclear whether *Mycobacterium w* is present in whole cell, disrupted cell, or fractionated cell form. According to the Examiner, the disclosure provides 10 exemplary *Mycobacterium w* compositions, but it is unclear which composition is claimed. The Examiner further argues that cancer treatment is an unpredictable art and that the disclosure fails to sufficiently support claims broadly drawn to methods of treating cancer, or methods to ameliorate the associated symptoms.

The rejection is traversed and reconsideration is respectfully requested.

The claims as amended recite that the *Mycobacterium w* is present in the compositions in heat killed whole cell, sonicated, solvent extract, or enzyme extract form. Thus the claims specify the particular form of the *Mycobacterium w*.

With respect to the dosage amounts and route and frequency of administration, applicants respectfully submit that one of ordinary skill in the art could determine proper dosage amounts and routes and frequency of administration of the claimed compositions without undue experimentation. The specification provides significant guidance with respect to these parameters. For instance, the specification provides 10 exemplary 0.1 mL dosage compositions comprising various *Mycobacterium w* preparations. Heat killed whole cell *Mycobacterium w* is used in compositions A-C and J, at cell counts ranging from  $0.5 \times 10^7$  –  $0.5 \times 10^9$ . Compositions D-I recite cellular extracts of *Mycobacterium w* obtained by subjecting  $1 \times 10^{10}$  cells to the various processing steps detailed in the specification at, e.g., page 10, lines 3-19.

The route of administration and frequency of administration of the dosage forms are clearly exemplified in the specification. See the specification at, e.g., page 11, lines 10-21, and page 12, lines 12-27. In the clinical study described in Example 4, Case 1, for example, a 0.1 mL dose was administered intradermally over the deltoid region at a frequency of once a week for three months. See the specification at page 11, lines 10-21. Example 5 teaches that a 0.1 mL dose administered intradermally at a frequency of once a month for two months is effective. See the specification at page 12, lines 12-27.

Furthermore, there are known techniques for determining an effective dose and route and frequency of administration. There is no reason to believe that such techniques would not be successful here.

Finally, applicants respectfully submit that the Examiner's assertion that the present specification only provides anecdotal evidence that the claimed compositions are an effective means for treating cancer is incorrect. The specification discloses human clinical trials conducted with a set of controls that demonstrate that the results correlate directly with the presence of the *Mycobacterium w* composition. See the specification at, e.g., page 13, line 20 to page 14, line 19. In Example 8(a), for example, breast cancer patents were effectively treated with *Mycobacterium w* and exhibited substantial improvement in symptoms compared to those in the control group. See the specification at, e.g., page 13, line 20 to page 14, line 3.

In view of the foregoing, applicants respectfully submit that the claims are enabled and request that this rejection be withdrawn.

### **Conclusion**

In view of the above amendments and remarks, it is respectfully requested that the application be reconsidered, and that the pending claims be allowed and the case passed to issue.

If there are any other issues remaining that the Examiner believes can be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

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## METHOD OF TREATING CANCER

### CROSS REFERENCE TO PRIOR APPLICATIONS

This application is a U.S. National Phase application under 35 U.S.C. § 371 of International Application No. PCT/IB02/05516, filed on February 15, 2007 and claims benefit to Indian Patent Application No. 1167MUM/2001, filed on December 10, 2001. The International Application was published in English on November 8, 2007 as WO 2003/049667 under PCT Article 21(2).

### BACKGROUND OF THE INVENTION

[0001] Cancer is believed to be caused by defective immune system. Many attempts have been made to improve immune system without success. Surprisingly it is found that ~~Mycobacterium~~ Mycobacterium containing compositions which are useful in improving immune status in patients with leprosy are also useful in management of cancer. They are found to be useful in decreasing burden of disease and reducing symptoms associated with cancer. More surprising was their synergy with conventional therapy in spite of fact that they work through entirely different mechanism. Still surprising was decrease in side effects of other therapy rather than increase in overall side effects in spite of use in same therapeutic amount along with increase in effect.

### PRIOR ART

[0002] Treatment of cancer has traditionally been approached through chemotherapy, coupled with radiotherapy for primary elimination of leukemias, neoplasms and ~~tumours~~ tumors. In contrast surgery has been used to remove solid ~~tumours~~ tumors. Therapy involves both curative and palliative leading to cure and reduction of suffering of the patient. ~~Immunotherapeutic~~ Immunotherapeutic methods have also been found to be effective against a restrictive range of ~~tumours~~ tumors of mesodermal origin suggesting that the immune system is capable of preventing or capable of delaying the growth of ~~tumours~~ tumors in certain cases.

[0003] Traditionally BCG vaccine is used for boosting of immunity of individuals with cancer. This has not been well accepted as a mode of therapy due to inconclusive results. The only accepted method of BCG is to use it for bladder cancer by way of intravesicular therapy. The disadvantage associated with use of BCG is development of systemic and local



tuberculosis caused by BCG. This is related to the fact that BCG contain live organism and they can be pathogenic to immunocompromised host.

[0004] U.S. Pat. No. 6,030,618 discloses an invention related to compositions, methods and kits for the prevention and treatment of primary and metastatic metastatic cancers and/or infectious diseases using heat shock/stress proteins (hsp) alone or in combination with each other and antigenic molecules to augment the immune responses to genotoxic and nongenotoxic factors, tumors, pathogens and infectious agents.

[0005] U.S. Pat. No. 5,767,156 provides a method of stimulating macrophage neutrophil and/or monocyte function in a subject. The method involves the administration of an effective amount of a free fatty acid having 18-24 carbon chain length with 2-6 double bonds and TNF or a TNF fragment or GM-CSF or interferon gamma.

[0006] U.S. Pat. No. 6,080,725 is directed to vaccines comprising one or more bacterial, viral, or ~~tumor~~ tumor-associated antigens; and one or more saponin-lipophile conjugate in which the lipophilic moiety such as a lipid, fatty acid, PEG, or terpene is covalently bonded to a non-acylated or deacylated triterpene saponin via a carboxyl group present on the 3-O-glucuronic acid of the triterpene saponin. The bacterial antigen in the vaccine are associated with a bacterial selected from diverse groups of bacteria including ~~Mycobacterium tuberculosis~~ Mycobacterium tuberculosis.

[0007] U.S. Pat. No. 6,221,351 B1 relates generally to ~~tumoricidal~~ tumoricidal compositions and methods and more specifically to superantigens or enterotoxins derived from Staphylococcus aureus, ~~Peptides~~ peptide ~~homologus~~ homologs to the ~~enterotoxins~~ enterotoxins including shock syndrome toxin, Streptococcal pyrogenic exotoxins, mycoplasma and mycobacterial species, minor lymphocyte stimulating antigens, heat shock proteins, stress peptides, mammary ~~tumor~~ tumor virus peptides, homologous synthetic polypeptides, biochemically derivatised enterotoxins, genetically engineered enterotoxins and fusion proteins. This invention also relates to superantigens expressed on the surface of lipid droplets in adjuvant-vehicle formulations or expressed in biologic cell surfaces as a result of enterotoxin gene transfection and used to produce a ~~tumoreicidal~~ tumoricidal response in ~~tumor~~ tumor bearing hosts. It also relates to enterotoxins and related compounds administered intravenously, subcutaneously, as in adjuvant form, or used extracorporeally in free or bound form to stimulate immunocytes, which are subsequently infused into ~~tumor~~ tumor bearing tissues.

[0008] U.S. Pat. No. 6,090,385 discloses a method of treating a cancer patient which comprises administering to said patient an anti-~~tumour~~ tumor effective amount of at least one of a water-soluble thermostable macromolecular antigen complex which is interspecific of microorganisms of the Mycobacteria, Nocardia, and Corynebacteria group and which exhibits after electrophoresis an ~~innumo-electrophoretic~~ immuno-electrophoretic precipitation pattern corresponding to that of the antigen complex 60 of the Mycobacterium bovis Clmetex Guerin Bacillus strain, or immunogenic fragments of such a complex. It comprises an additional step of administering a therapeutic agent specific against the patient's cancer.

[0009] U.S. Pat. No. 6,067,964 suggests the delaying or preventing the growth or spread of breast or bronchial neoplasm which comprises administering to a subject in need of the same, antigenic and/or immunoregulatory material which comprises killed cells of Mycobacterium vaccae strain NCTC 11659 in an amount sufficient at least to delay or prevent the growth or spread of said neoplasm. This could be administered by intradermal injection.

[0010] U.S. Pat. No. 6,033,669 describes a method of stimulating the generation of cytotoxic T Cells (CTLs) in a patient, wherein the CTLs have the potential to destroy or attenuate cells presenting a characteristic disease-associated ~~carbohydrate~~ carbohydrate structure, which comprises administering to the patient an effective dose of a peptide/carbohydrate conjugate complex capable of generating cytotoxic T cell immunity against a ~~carbohydrate~~ carbohydrate structure, said conjugate structure comprising (i) a peptide component capable of binding an MH class I molecule, and (ii) a carbohydrate component comprising of immunogenic specificity of said disease-associated carbohydrate structure and being of a size that enables a T cell receptor to encompass an epitope of said disease-associated carbohydrate structure. This is claimed to be effective for treatment of melanoma, breast cancer, lung cancer or gastrointestinal cancer.

[0011] It has surprisingly been found that pharmaceutical compositions containing ~~Mycobacterium W~~ Mycobacterium w (Mw) are effective in the treatment of a broad range of cancer indications. Pharmaceutical compositions as per present invention may contain extracts of ~~Mycobacterium W~~ Mycobacterium w alone or in combination of ~~Mycobacterium W~~ Mycobacterium w. As per another aspect of present invention pharmaceutical composition may contain other immunomodulator. It can be administered in various ways including intradermal, oral, intravesical etc. The present invention discloses such formulations and the method of their manufacture and use.

[0012] ~~Mycobacterium w~~ *Mycobacterium w* is a rapidly growing Mycobacterium which is not a pathogen.

[0013] ~~Mycobacterium w~~ *Mycobacterium w* is a non-pathogenic, cultivable, atypical mycobacterium, with biochemical properties and fast growth characteristics resembling those belonging to Runyons group IV class of Mycobacteria in its metabolic and growth properties but is not ~~identical~~ identical to those strains currently listed in this group. It is therefore thought that (~~M<sub>w</sub>~~) M<sub>w</sub> is an entirely new strain. The species identity of ~~M<sub>w</sub>~~ M<sub>w</sub> has been defined by polymerase chain reaction DNA sequence determination.

[0014] It has been found to share antigens with Mycobacterium leprae and Mycobacterium tuberculosis. It is found to provide prophylaxis against leprosy in humans by converting lepromin negative individuals to lepromin positivity. It is also found to provide prophylaxis against tuberculosis in animals. In leprosy it is also found to reduce duration of therapy for bacterial killing, clearance as well as clinical cure when used along with multi drug therapy.

#### SUMMARY OF THE INVENTION:

[0015] According to present invention, a pharmaceutical composition made from ~~Mycobacterium w~~ (~~M<sub>w</sub>~~) *Mycobacterium w* (M<sub>w</sub>) is found to be useful in the management of cancer. We have now found that the same therapeutic agent is useful in management of cancer. The use of ~~Mycobacterium w~~ *Mycobacterium w* containing formulations is associated with decrease in burden of cancer tissue, decreasing symptoms associated with cancer and improving quality of life. It also improves tolerance to other therapies.

[0016] Therapeutic agent which may be used in the present invention resembles M<sub>w</sub> and non-pathogenic, cultivable, atypical mycobacterium, with biochemical properties and fast growth characteristics resembling those belonging to Runyons group IV class of Mycobacteria in its metabolic and growth properties but is not identical to those strains currently listed in this group. It is therefore thought that (~~M<sub>w</sub>~~) M<sub>w</sub> is an entirely new strain.

[0017] The species identity of M<sub>w</sub> has been defined by polymerase chain reaction DNA sequence determination and differentiated from thirty other species of mycobacteria. It however differs from those presently listed in this group in one respect or the other. By base sequence analysis of a polymorphic region of pattern analysis, it has been established that M<sub>w</sub> M<sub>w</sub> is a unique species distinct from many other known mycobacterial species examined which are: M. avium, M. intracellulare, M. scrofulaceum, M. kansasii, M. gastri, M. goodii, M. gordonae,

M. shimoidel, M. Malmoeense, M. haemophilum, M. terrae, M. nonchromogenicum, M. trivale, M. marinum, M. flavescens, M. simian, M. szulgai, M. xenopi, M. asiaticum, M. aurum, M. amegris, M. vaccae, M. fortuitum subsp. fortuitum, M. fortuitum subsp. Peregrinum, M. chelonae subsp. Chelonae, M. chelonae subsp. Abscessus, M. genavense, M. tuberculosis, M. tuberculosis H<sub>37</sub>R<sub>v</sub>, M. paratuberculosis.

[0018] The object of the present invention is to provide a pharmaceutical composition containing ~~Mycobacterium w~~ Mycobacterium w (Mw) for the treatment of cancer.

[0019] Another object of the present invention is to provide a pharmaceutical composition containing ~~Mycobacterium w~~ Mycobacterium w (Mw) to improve quality of life in patient suffering from cancer.

[0020] Yet another object of the invention is to provide a ~~pharmaceutical~~ pharmaceutical composition derived from ~~mycobacterium w~~ Mycobacterium w that are useful for the management of cancer.

[0021] Yet another object of the invention is to provide a pharmaceutical composition derived from ~~Mycobacterium w~~ Mycobacterium w to provide symptomatic relief for patients suffering from cancer.

[0022] Yet another object of present invention is to provide a pharmaceutical composition which decreases side effects of standard therapy like radiotherapy, chemotherapy.

[0023] Yet another object of present invention is to provide an a pharmaceutical composition containing ~~Mycobacterium w~~ Mycobacterium w (Mw) which decreases burden of cancer cells/tissues of primary and/or secondary (metastatic), sensitive and/or refractory to conventional treatment.

[0024] Yet another object of present invention is to provide a pharmaceutical composition which improves effect of conventional therapies.

#### BRIEF DESCRIPTION IF THE DRAWINGS

[0025] FIG. 1 is Xray non small cell lung cancer before treatment--subject 1

[0026] FIG. 2 is Xray non small cell lung cancer after treatment--subject 1

[0027] FIG. 3 is Xray non small cell lung cancer before treatment--subject 2

[0028] FIG. 4 is Xray non small cell lung cancer after treatment--subject 2

[0029] FIG. 5 is CT Scan report of patient operated for colorectal cancer with liver metastasis before treatment

[0030] FIG. 6 is CT Scan report of patient operated for colorectal cancer with liver ~~metastasis~~ metastasis after treatment

DETAILED DESCRIPTION OF THE INVENTION:

[0031] In accordance with the invention the composition of a pharmaceutical composition the method of preparation, HPLC characteristic its safety and tolerability, methods of use and outcome of treatments are described in following examples. The following are illustrative examples of the present invention and scope of the present invention should not be limited by them.

Example 1

[0032] The ~~pharmaceutical~~ pharmaceutical compositions:

A. Each dose of 0.1 ml of therapeutic agent contains:

Mycobacterium w., (heat killed)	$0.50 \times 10^9$
Sodium Chloride I.P.	0.90% w/v
Tween 80	0.1% w/v
Thiomersal I.P. (As a Preservative)	0.01% w/v
Water for injection I.P.	q.s. to 0.1 ml

## -continued

<u>B. Each dose of 0.1 ml of therapeutic agent contains:</u>	
Mycobacterium w., (heat killed)	0.50 × 10 <sup>9</sup>
Sodium Chloride I.P.	0.90% w/v
Triton × 100	0.1% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>C. Each dose of 0.1 ml of therapeutic agent contains:</u>	
Mycobacterium w., (heat killed)	0.50 × 10 <sup>8</sup>
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>D. Each dose of 0.1 ml of therapeutic agent contains</u>	
Extract of Mycobacterium w	
after sonication from	
1 × 10 <sup>10</sup> Mycobacterium w	
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>E. Each dose of 0.1 ml of therapeutic agent contains</u>	
Methanol Extract of 1 × 10 <sup>10</sup>	
Mycobacterium w	
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>F. Each dose of 0.1 ml of therapeutic agent contains</u>	
Chloroform Extract of 1 × 10 <sup>10</sup>	
Mycobacterium w	
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>G. Each dose of 0.1 ml of therapeutic agent contains</u>	
Acetone Extract of 1 × 10 <sup>10</sup>	
Mycobacterium w	
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>H. Each dose of 0.1 ml of therapeutic agent contains</u>	
Ethanol Extract of 1 × 10 <sup>10</sup>	
Mycobacterium w	
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>I. Each dose of 0.1 ml of therapeutic agent contains</u>	
Lipase Extract of 1 × 10 <sup>10</sup>	
Mycobacterium w	
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a preservative)	
Water for injection I.P.	q.s. to 0.1 ml
<u>J. Each dose of 0.1 ml of therapeutic agent contains</u>	
Mycobacterium w (heat killed)	0.5 × 10 <sup>7</sup>
Extract of mycobacterium w obtained	
1 × 10 <sup>9</sup> Mycobacterium w by	
disruption, solvent extraction	
or enzymatic extraction.	
Sodium Chloride I.P.	0.90% w/v
Thiomersal I.P.	0.01% w/v
(As a Preservative)	
Water for injection I.P.	q.s. to 0.1 ml

## Example 2

The process of preparing a pharmaceutical composition

[0033] A. Culturing of ~~Mycobacterium w~~ Mycobacterium w.

[0034] i) Preparation of ~~culture~~ culture medium. ~~Mycobacterium w~~ Mycobacterium w is cultured on solid medium like L J medium or liquid medium like middle brook medium or sauton's liquid medium. For better yield middle brook medium is enriched. It can be preferably enriched by addition of glucose, bactotryptone, and BSA. They are used in ratio of 20:30:2 preferably.

[0035] The enrichment medium is added to middle brook medium. It is done preferably in ratio of 15:1 to 25:1 more ~~preferably~~ preferably in ratio of 20:1.

[0036] ii) Bioreactor operation

[0037] a) Preparation of vessel

[0038] The inner contact parts of the vessel (Joints, mechanical seals, o-ring/gasket grooves, etc.) should be properly cleaned to avoid any contamination. Fill up the vessel with 0.1 N NaOH and leave as such for 24 H to remove pyrogenic materials and other contaminants. The vessel is then cleaned first with acidified water, then with ordinary water. Finally, the vessel is rinsed with distilled water (3 times) before preparing medium.

[0039] b) ~~Sterilization~~ Sterilization of bioreactor

[0040] The bioreactor containing 9L distilled water is sterilized with live steam (indirect). Similarly the bioreactor is sterilized once more with Middlebrook medium. The other addition bottles, inlet/outlet air filters etc. are autoclaved (twice) at 121° C for 15 minutes. Before use, these are dried at 50° C oven.

[0041] c) Environmental parameter

[0042] i. Temperature:  $37 \pm 0.5$  degree. C.

[0043] ii. pH: 6.7 to 6.8 initially.

[0044] B. Harvesting and Concentrating

[0045] It is typically done at the end of 6<sup>th</sup> day after culturing under aseptic condition. The concentration of cells (~~palletisation~~ pelletization) is done by centrifugation.

[0046] C. Washing of Cells

[0047] The ~~pallet~~ pellet so obtained is washed minimum three times with normal saline. It can be washed with any other fluid which is preferably isotonic.

[0048] D. Adding Pharmaceutically Acceptable Carrier

[0049] Pyrogen free normal saline is added to ~~pallet~~ pellet. Any other pyrogen free isotonic fluid can be used as a pharmaceutical carrier. The carrier is added in amount so as get to desired concentration of active in final form.

[0050] E. Adding Preservative

[0051] To keep the product free from other contaminating bacteria for its self life preservative is added. Preferred preservative is thiomersol which is used in final concentration of 0.01% w/v.

[0052] F. Terminal Sterilization

[0053] Terminal sterilization can be done by various physical methods like application of heat or ionizing radiation or sterile filtration.

[0054] Heat can be in the form of dry heat or moist heat. It can also be in the form of boiling or ~~pasterisation~~ pasturization.

[0055] Ionizing radiation can be ultraviolet or gamma rays or microwave or any other form of ionizing radiation.

[0056] It is preferable to autoclave the final product.

[0057] This can be done before after filling in a final package.

[0058] G. Quality Control

[0059] i. The material is evaluated for purity, sterility.

[0060] ii. The organisms are checked for acid fastness after gram staining.

[0061] iii. Inactivation test: This is done by culturing the product on L J medium to find out any living organism.

[0062] iv. Pathogenicity and/or contamination with pathogen.

[0063] The cultured organisms are infected to Balb/c mice. None of the mice should die and all should remain healthy and gain weight. There should not be any macroscopic or microscopic lesions seen in liver, lung spleen or any other organs when animals are killed up to 8 weeks following treatment.

[0064] v. Biochemical Test:

[0065] The organism is subjected to following biochemical tests:

[0066] a) Urease

[0067] Tween 80 hydrolysis

[0068] Niacin test

[0069] Nitrate reduction test



[0070] The ~~organism~~ organism gives negative results in urease, ~~tween~~ Tween 80 hydrolysis and niacin test. It is positive by nitrate reduction test

[0071] H. Preparation of ~~Constituents~~ Constituents of ~~Mycobacterium-w~~ Mycobacterium w.

[0072] The constituents of ~~Mycobacterium-w~~ Mycobacterium w can be prepared for the purpose of invention by:

[0073] Cell disruption

[0074] II. Solvent extraction

[0075] III. Enzymatic extraction

[0076] The cell disruption can be done by way of ~~sonietion~~ sonication or use of high pressure fractionometer or by application of osmotic pressure ingredient.

[0077] The solvent extraction can be done by any organic solvent like chloroform, ~~ethanal~~ ethanol, methanol, acetone, phenol, isopropyl alcohol, ~~acetic~~ acetic acid, urea, hexane etc.

[0078] The enzymatic extraction can be done by enzymes which can digest cell wall/membranes. They are typically proteolytic in nature. Enzyme liticase and pronase are the preferred enzymes. For the purpose of invention cell constituents of ~~Mycobacterium-w~~ Mycobacterium w can be used alone in place of ~~mycobacterium-w~~ Mycobacterium w organisms or it can be added to the product containing ~~mycobacterium-w~~ Mycobacterium w.

[0079] Addition cell constituents results in improved efficacy of the product.

Example 3

~~Characterisite~~ Characteristics of Constituents of ~~Mycobacterium-w~~ Mycobacterium w by HPLC Analysis

[0080] The constituents of ~~mycobacterium-w~~ Mycobacterium w used for the purpose of invention when subjected to HPLC analysis gives a single peak at 11 minutes. No other significant peaks are found beyond. The peak is homogenous and devoid of any notch suggesting homogeneity of material obtained

[0081] HPLC analysis was done using a waters system high performance liquid chromatography apparatus.

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Column:	Novapak c1860A, 4 $\mu$ m, 3.9 $\times$ 150 mm.
The guard column:	Novapak c18
Column Temperature:	30° C.
Flow rate:	2.5 ml/min
Injection volume:	25 $\mu$ L.
Mobile phase:	

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Solvent A:  
HPLC grade methanol.  
Solvent B:  
HPLC grade methylene chloride

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[0082] Binary gradient:

[0083] The HPLC gradient initially comprised 98% (v/v) methanol (solvent B). The gradient was increased linearly to 80% A and 20% B at one minute; 35% A and 65% B at 10 minutes, held for 5 seconds and then decreased over 10 seconds back to 98% A and 2% B.

#### Example 4

#### Management of Cancer Refractory to Standard Treatment

[0084] CASE 1

[0085] A 70 year old female suffering from multiple myeloma was receiving malphalan and prednisolone a therapy for 5 years. The disease recurred with bone pain. Her general condition was poor and she was bedridden. Her hemoglobin was reduced to 5.5 gm. She was put on intradermal injection of a pharmaceutical composition injection of a pharmaceutical composition containing ~~mycobacterium w~~ *Mycobacterium w* was per present invention. It was given as 0.1 ml intradermally over deltoid region at the interval of one week. At the end of 3 months she is symptoms general condition has improved drastically and she is able to walk on her own. Her hemoglobin value has risen to 7.7 gm/dl from 5.5 gm/dl in absence of any specific treatment on anaemia.

[0086] CASE 2

[0087] A 50 year old postmenopausal woman under went lumpectomy for a fungating mass in her last breast (carcinoma breast T<sub>4</sub>, N<sub>1</sub> M<sub>1</sub>). The tumor was hormone independent and receptor status for estrogen and progesterone was negative. Following surgery she developed cough and breathlessness. It was found to be due to large metastatic lesion in her chest. A pharmaceutical composition as per present invention was added to her therapy. At the end of the three months, there was a remarkable improvement in her cough and breathlessness. X-ray chest showed 25% decrease in size of metastatic lesion. The ~~mycobacterium w~~

Mycobacterium w containing pharmaceutical ~~composition~~ composition as per present invention was administered intradermally over deltoid region.

[0088] CASE 3

[0089] A 68 year old male suffering from carcinoma esophagus-midthird had received radiotherapy and was on chemotherapy (one cycle completed) He developed dysphagia due to progress of disease. He also had ~~neutropenia~~ neutropenia with fall in total WBC count. Therapy with a pharmaceutical composition was started. It resulted in improvement in his symptoms gradually. At the end of three months. The swallowing became normal with improvement in general condition and normalization of WBC count. The pharmaceutical composition as per present invention was given as 0.1 ml intradermally at weekly interval 2<sup>nd</sup> dose was delayed and administered at the interval of 15 days instead of 1 week. It comprised of 0.3 ml instead of 0.1 ml.

[0090] In case 1 and 3 improvement was seen in spite of absence of chemotherapy while in case 2 chemotherapy (FAC) also continued.

[0091] Thus this cases illustrates that pharmaceutical compositions containing ~~Mycobacterium w~~ Mycobacterium w (Mw) as per present invention are useful in treatment of cancer which are refractory to standard therapy. Their useful is associated with amelioration of symptoms, improvement in general well being and quality of life, improvement in other associated conditions like anemia, neutropenia.

#### Example 5

Effect of ~~Pharmaceutical~~ Of Pharmaceutical Composition On Cancer When Used Alone

[0092] Superficial bladder cancer presents as hematuria. It is amenable to various forms of therapy. Drugs used to achieve remission are given intravesically e.g. doxorubicin or BCG.

[0093] In four patients with superficial bladder cancer diagnosed cystoscopically pharmaceutical composition as per present invention was given intradermally. It was given as 0.1 ml every month. By six weeks (after two injections) everybody became asymptomatic. Eight weeks later cystoscopy was performed. Surprisingly it was found that there was absence of any detectable lesion cystoscopically. Six months follow-up did not reveal any recurrence of symptoms. Cystoscopy also revealed normal bladder mucosa with absence of detectable lesion.

[0094] Thus findings are suggestive of effect of pharmaceutical composition containing ~~mycobacterium w~~ Mycobacterium w as effective therapy in management of bladder cancer when given intradermally over deltoid region.

#### Example 6

Effect of ~~Myco~~Myco~~bacterium w~~ bacterium w When Radiotherapy Is Not Adequate

[0095] Muscle invasive bladder cancer.

[0096] Muscle invasive bladder cancer (T<sub>4</sub>) can be managed by radical cystectomy. However it is desirable to preserve bladder. Radiotherapy and/or ~~chemotherapy~~ chemotherapy are not adequate in achieving local control/remission of disease.

[0097] Five patients with muscle invasive bladder cancer were treated by intradermal injection of ~~Myco~~Myco~~bacterium w~~ bacterium w over both deltoid. The intradermal ~~Myco~~Myco~~bacterium w~~ bacterium w was repeated every month on any on deltoid for six months. All received standard radiotherapy for a total of 71 Gy.

[0098] At the end of two months all were symptom free. Cystoscopy and computerized axial tomography (T scan) failed to reveal any detectable lesion suggesting complete remission of disease. All are disease free after a follow-up of 8 months or longer after beginning therapy.

[0099] Thus ~~mycobacterium w~~ Mycobacterium w is effective in achieving complete remission and maintaining it.

[0100] No side effects were noticed by any of the patients.

#### Example 7

Effect of ~~Myco~~Myco~~bacterium w~~ bacterium w When Chemotherapy Is Not Adequate

[0101] Non small cell lung cancer.

[0102] Non small cell lung cancer is difficult to manage. It usually does not respond well to chemotherapy, or radiotherapy. The response rate is inversely proportionate to extent of disease. In disease with extent T<sub>4</sub> surgery is not indicated and chemotherapy and/or radiotherapy has hardly any effect and carries poor prognosis.

[0103] Thus findings of this study suggests that ~~mycobacterium w~~ Mycobacterium w has significant effect on difficult to treat cancer.

#### Example 8

Effect of ~~Myco~~Myco~~bacterium w~~ bacterium w Containing Therapy On Quality of Life and Side Effects of Chemotherapy

[00104] a) Carcinoma breast with bone metastasis.

[00105] In a controlled study involving 20 patients with a breast cancer and bone metastasis effect of ~~mycobacterium w~~ Mycobacterium w was evaluated. All patients received chemotherapy in the form of cyclophosphamide, adriamycin and 5-fluorouracil. ~~Myecobacterium w~~ Mycobacterium w containing compositions were given as intradermal injections of 0.1 ml every week for two months followed by every 15 days for two months and monthly for two months for a total duration six months. 10 patients of 20 randomly received it while remaining 10 were kept as controls.

[00106] None of the patients in treatment group developed ~~diarrhoea~~ diarrhea and vomiting compared to 8 of 10 patients in control group. Mucositis was seen in 1 patient in treatment group compared to 5 patients in control group. Bone marrow depression as manifested by leucopenia, thrombocytopenia and anemia was seen in 2 patients in treatment group and 6 patients in control group. There was increase in weight in 3 patients in treatment group while none of the patients showed increase in weight. All patients had remarkable improvement in quality of life in treatment group which was not seen in any one in control group.

[00107] b) Head and Neck Cancer

[00108] In a controlled study in 20 patients with histologically proven advanced head and neck cancer with minimum of 6 months expectancy effect of ~~mycobacterium w~~ Mycobacterium w was evaluated. Each patient received chemotherapy containing ~~cisplatin~~ Cisplatin and 5-FU. ~~Myecobacterium w~~ Mycobacterium w was given to randomly selected 10 patients as 0.1 ml intradermally over deltoid region every 15 days for 3 months. The first dose was given as 0.2 ml divided over two deltoid region.

[00109] The chemotherapy induced hematological side effects resulted in postponement of chemotherapy in 2 out of 10 patients in treatment group and 5 out of 10 patients in control group.

[00110] Mucositis was evident on day 15 after chemotherapy. It was seen in 2 patients in treatment group and 6 patients in control group.

[00111] Nausea/vomiting was seen in all patients in control group while in none in treatment group.

[00112] Thus use of ~~mycobacterium w~~ mycobacterium w was useful in reducing side effects of chemotherapy.

Example 9

Use of ~~Mycobacterium w~~ Mycobacterium w In Terminally Sick Patients With Cancer

[00113] A 65 year old male patient was diagnosed to have carcinoma pancreas with metastasis in liver and lung. He was judged to be terminally ill with incurable cancer was not offered any treatment. He developed extensive cough and breathlessness and was unable to sleep. He was administered ~~Mycobacterium w~~ Mycobacterium w 0.3 ml intradermally biweekly. Within 10 day his cough was controlled and general condition showed improvement. He started doing all his routine work by himself and started getting normal sleep. If for some reason administration of ~~Mycobacterium w~~ Mycobacterium w was delayed beyond 4 days there used to be recurrence of cough and disturbances in sleep. He lived for 14 weeks after therapy and died natural death. X-ray chest taken before and two months after showed some improvement in lesion. He did not receive any other therapy all throughout.

[00114] The ~~Mycobacterium w~~ Mycobacterium w was ameliorating symptoms in terminally ill patient due to cancer.